AMENDMENTS TO THE SPECIFICATION:

Please replace the first paragraph on page 1, lines 9-11 of the Specification with the following amended paragraph:

The present invention relates to a laser scan type fluorescence microscope used for a fluorescence observation or a ~~co-focus point~~ confocal fluorescence observation in ~~application of an~~ applications such as function elucidation ~~of the function~~ or ~~an~~ imaging of ~~a cell and the like~~ cells.

Please replace the paragraphs on page 1, line 19 through page 5, line 26 with the following amended paragraphs:

[0003]

Fig. 1 is an outline block diagram showing one conventional example of a laser scan type ~~co-focal point~~ confocal fluorescence microscope.

The laser scan type ~~co-focal point~~ confocal fluorescence microscope of Fig. 1 comprises a laser light source section 51, an objective ~~lens~~ optical system 53 which condenses excitation light from a laser light source section 51 on a sample 52, a scanning means 54 which ~~makes~~ scans a surface of the sample 52 with the excitation light from the laser light source section 51 ~~scan on a surface of the sample 52~~, a pupil projection lens 55 arranged between the scanning means 54 and the objective ~~lens~~ optical system 53, a detection optical system 56 for detecting fluorescence which ~~is emanated~~ emanates from the sample 52 and ~~has penetrated~~ passes the objective ~~lens~~ optical system 53 and the pupil projection lens 55.

The laser light source section 51 has a laser light source 51a ~~and~~, a collimating optical system ~~which consists of~~ including lenses 51b and 51d and a pinhole 51c, and a dichroic mirror 51e.

The objective ~~lens~~ optical system 53 has an objective lens 53a and an image forming lens 53b for forming an intermediate image of ~~an objective lens 53a and~~ the sample 52. Moreover, a ~~backside~~ back focal position of the objective lens 53a is ~~constituted so that it may become~~ made conjugate ~~at~~ with a position near the scanning means 54 by the image forming lens 53b and the pupil projection lens 55.

The scanning means 54 ~~consists of~~ is configured as a proxy type Galvano mirror having Galvano mirrors 54a and 54b.

The detection optical system 56 has a dichroic mirror 56a, a barrier filter 56b, a lens 56c, and a ~~co-focal point~~ confocal pinhole 56d and a light receiving optical sensor 56e, such as a photomultiplier ~~and the like~~.

Furthermore, the microscope of Fig. 1 has a dichroic mirror 57 which leads the fluorescence from the sample 52 to the detection means 56 while leading the excitation light from the light source section 51 to the sample 52, a mirror 59 which deflects the light ~~which~~ transmitted through the pupil projection lens 55 to the image forming lens 53b, an ~~eye-piece~~ eyepiece optical system 60 for observing the image of the sample 52, and a fluorescence lighting optical system 61 used ~~at the time of a~~ in normal fluorescence observation.

[0004]

Thus, in the laser scan type ~~co-focal point~~ confocal fluorescence microscope as constituted in Fig. 1, the excitation light ~~emanated~~ emanating from the laser light source 51a is condensed at the pinhole 51c by the lens 51b, and then is converted into a beam of parallel ~~light~~ rays by the lens 51d. Then, this excitation light is led to the proxy type Galvano mirror section ~~that~~, which is the scanning means 54 ~~through~~, via dichroic mirrors 51e and 57, and the ~~luminous flux~~ beam of rays of it is shifted ~~to direction of~~ two ~~dimensions~~ dimensionally in reference to the optical axis by each rotation of Galvano mirrors 54a and 54b ~~to an optical axis, and it is condensed~~, to be formed as a primary image as being focused on the intermediate image position 58 through the pupil projection lens 55~~, and thus an primary image is formed~~. The excitation light condensed ~~to~~ at the intermediate image position 58 is ~~irradiated to~~ incident on the sample 52 ~~in~~ at a minute spot ~~like shape through~~ via the mirror 59, the image forming lens 53b, and the objective lens 53a. At this time, the surface of the sample 52 is scanned with the excitation light ~~irradiated by the surface 52 of the sample is scanned~~ by the scanning means 54.

[0005]

The ~~backside~~ back focal position of the objective lens 53a is projected by the image forming lens 53b and the pupil projection lens 55 near the proxy type Galvano mirror which is the scanning means 54.

Fluorescence excited on the sample 52 by ~~irradiating~~ irradiation with the excitation light~~,~~ is led to the detection optical system 56 ~~through~~ via the objective lens 53a, the image forming lens 53b, the pupil projection lens 55, the scanning means 54, and the dichroic mirror 57. Then, a wavelength separation is carried out by the dichroic mirror 56a, and only ~~the~~ fluorescence ~~which transmitted~~ that passes through the ~~co-focal point~~ confocal pinhole

~~through~~ via the barrier filter 56b and the lens 56c is detected by the light receiving optical sensor 56e, such as a photomultiplier.

[0006]

In carrying out a normal fluorescence observation through the ~~eye~~ eyepiece optical system 60, a fluorescence lighting optical system 61 equipped with a different light source 61a from the laser light source 51a is used. Excitation light ~~emanated~~ emanating from the light source 61a is transmitted through a lens 61b and a filter 61c, ~~and~~ is reflected by the ~~dichroic mirror~~ dichroic mirror 61d, and illuminates the sample 52 through the ~~objective lens~~ objective lens 53a. Fluorescence excited on the sample 52 by ~~irradiating~~ irradiation with the excitation light is condensed by the ~~objective lens~~ objective lens 53a, ~~and~~ is subjected to wavelength separation ~~is carried out~~ by the ~~dichroic mirror~~ dichroic mirror 61d arranged ~~at~~ in the fluorescence lighting optical system 61, and ~~it~~ is observed ~~through~~ via the prism 60a ~~of the eyepiece optical system 60,~~ and the eyepiece 60b of the eyepiece optical system 60 through the barrier filter 61e.

~~DISCLOSURE OF THE INVENTION~~

[0007]

Such a conventional laser-scan-type ~~co-focal point~~ confocal fluorescence microscope is excellent in resolution, and it has an advantage that light from other than a minute spot to be observed can be eliminated. Thus, it is useful for carrying out an intracellular functional elucidation etc.

However, in the laser scan type ~~co-focal point~~ confocal fluorescence microscope, the equipment itself becomes large since it is necessary to add an optical system such as a pupil projection lens 55 and a ~~scanning means~~ scanning means 54 mentioned above etc., in addition to an optical system used for a normal fluorescence observation, such as an objective lens 53a and an image forming lens 53b~~,~~.

[0008]

That is, generally as for the optical system of a laser-scan type ~~co-focal point~~ confocal fluorescence microscope, the focal length of an image forming lens ~~has become~~ is such long ~~such~~ as around 180mm. ~~For this reason~~ Consequently, a total length from a sample to the scanning means arranged near a conjugate position of a pupil of an objective lens becomes 400-500mm, ~~and~~ to enlarge the whole equipment ~~becomes enlarged~~.

[0009]

For this reason, a ~~co-focal point~~ confocal fluorescence observation and a fluorescence observation become possible only in case that the sample is arranged on a stage of a microscope.

Moreover, when a ~~co-focal point~~ confocal fluorescence observation is actually performed to a rat, a small animal or a cell under a cultivation environment where it is alive (in vivo), there is a restriction that the observation environment must be built on the stage. Furthermore, a laser scan type ~~co-focal point~~ confocal fluorescence microscope is generally ~~constituted so that it may observe~~ designed to perform observation in a state where the optical axis of an objective lens becomes perpendicular to a surface of the stage. Therefore, it is difficult to observe the sample from a slant direction. Moreover, it is difficult to ~~observe by~~ perform observation upon leaning the whole laser scan type ~~co-focal point~~ confocal fluorescence microscope to the sample~~,~~ or ~~to observe by~~ leaning the sample and the stage.

BRIEF SUMMARY OF THE INVENTION

[0010]

The present invention is made in view of problems mentioned above, and it aims at providing a laser scan type fluorescence microscope~~, which can be miniaturized~~ that is small in size compared with the conventional laser scan type ~~co-focal point~~ confocal fluorescence microscope~~,~~ and that can ~~observe~~ achieve observation in a state ~~that~~ where a cell is alive (in ~~vive~~ vivo) ~~by~~ with wavelengths from a visible region to a near-infrared region with sufficient user-friendly operation.

[0011]

In order to attain the above-mentioned purpose, the laser scan type fluorescence microscope according to the present invention comprises a laser light source section, an objective ~~lens~~ optical system which condenses excitation light from the laser light source section on a sample, a scanning means which makes the excitation light from the laser light source section scan on a surface of the sample, a pupil projection lens arranged between the scanning means 54 and the objective ~~lens~~ optical system, and a detection optical system for detecting fluorescence which ~~is emanated~~ emanates from the sample and ~~has penetrated~~ passes the objective ~~lens~~ optical system and the pupil projection lens. The objective ~~lens~~ optical system has an objective lens~~,~~ and an image forming lens for forming an intermediate image of the sample, ~~and~~ a ~~backside~~ back focal position of the objective lens ~~constituted so that it may become~~ is made conjugate ~~at~~ with a position near the scanning means by the image forming lens and the pupil projection lens, and the following condition (1) is satisfied~~;~~:

$$0.15 \leq D/L \leq 0.5 \quad \ldots(1)$$

where D is a ~~co-focal length~~ parfocal distance of the objective lens, and L is a distance from the sample surface to the ~~conjugate~~ position ~~of~~ conjugate with the ~~backside~~ back focal position of the objective lens and arranged near the scanning means.

Please replace the paragraph on page 6, lines 4-13 with the following amended paragraph:

[0013]

In the laser scan type fluorescence microscope according to the present invention, the pupil projection lens ~~consists of~~ includes two or more lens groups, wherein ~~a concave surface of~~ a lens surface arranged ~~at the~~ nearest ~~side of~~ the scanning means is ~~directed~~ concave toward the scanning-means side, and ~~a concave surface of~~ a lens surface arranged ~~at an~~ nearest the intermediate image ~~side~~ is ~~directed~~ concave toward the intermediate image side, and the following condition (2) is satisfied~~;~~:

$$0.2 \leq Fe/D3 \leq 0.5 \quad \ldots(2)$$

where D3 is a distance from the ~~conjugate~~ position ~~of~~ that is conjugate with the pupil of the objective lens and is located near the scanning means to the intermediate image position of the image forming lens, and Fe is a focal length of the pupil projection lens.

Please replace the paragraphs on page 6, line 25 through page 7, line 22 with the following amended paragraphs:

[0015]

The laser scan type fluorescence microscope according to the present invention is characterized in that the image forming lens ~~consists of~~ includes two lens groups ~~having~~ that are a front group ~~at the side of an~~ arranged on the intermediate image side and a rear group ~~at the side of an~~ arranged on the objective lens side, ~~and the lens group of~~ the front group of the image forming lens has at least one negative lens, and the following conditions (5) and (6) are satisfied~~;~~:

$$0.4 \leq D2/FTL \leq 1 \quad \ldots(5)$$

$$0.7 \leq FTL1/FTL \leq 1.5 \quad \ldots(6)$$

where FTL1 is a focal length of the rear group of the image forming lens, and D2 is an interval between the front group of the image forming lens and the rear group of the image forming lens.

[0016]

The laser scan type fluorescence microscope according to the present invention comprises a first multi-mode fiber which leads the excitation light from the laser light source section to the scanning means, a second multi-mode fiber which leads the fluorescence from the sample to the detection optical system, a first lens by which entry of the excitation light to the first multi-mode fiber is carried out, and a second ~~multi-mode fiber~~ lens by which ~~leads~~ entry of the fluorescence from the sample to the detection optical system is carried out, and the following conditions (7) to (9) are satisfied~~;~~:

$$2 \leq \Phi em/\Phi ex \leq 12 \quad ...(7)$$

$$0.61 \times (\gamma ex/\text{~~Na~~}NAex) < \Phi ex \quad ...(8)$$

$$0.61 \times (\lambda em/NAem) < \Phi em \quad ...(9)$$

where Φex is a diameter of a core of the first multi-mode fiber, Φem is a diameter of a core of the second multi-mode fiber, NAex is ~~an~~ a numerical aperture ~~number by which~~ where entry of the excitation light to the first multi-mode fiber by the first lens is carried out, λex is a wavelength of the excitation ~~wavelength~~ light, NAem is ~~an~~ a numerical aperture ~~number by which~~ where entry of the fluorescence to the second multi-mode fiber by the second lens is carried out, and λem is a wavelength of the fluorescence ~~wavelength~~.

Please replace the paragraphs on page 8, lines 4 through page 23, line 1 with the following amended paragraphs:

[0019]

By arranging the optical transmission means between the laser optical system and the scanning means, a degree of freedom can be given to arrangement of the optical system ~~of~~ in the main body that is from the scanning means to the objective lens, and of the laser optical system, and a miniaturized optical system ~~of~~ in the main body suitable for observation in a state where the sample is alive (in vivo) can be provided.

[0020]

By arranging ~~such that~~ the pupil projection lens, which relays ~~parallel luminous flux~~ a beam of parallel rays deflected by the scanning means to an intermediate image position ~~has~~,to include two or more lens groups~~, a concave surface of~~ with a lens surface arranged ~~at the~~ nearest ~~side of~~ the scanning means ~~is directed~~ being concave toward the scanning means side~~,~~ and ~~a concave surface of~~ a lens surface arranged ~~at the~~ nearest ~~side of~~ the intermediate image ~~side is directed~~ being concave toward the ~~middle~~ intermediate image side, correction of ~~an~~ optical performance in the intermediate image can be carried out well.

By satisfying the condition (2), ~~shortening of a distance from the scanning means to the intermediate image position, and~~ miniaturization of the equipment can be achieved with a shortened distance from the scanning means to the intermediate image position, while ~~making an~~ good optical performance of the pupil projection lens ~~good~~ is achieved.

[0021]

By satisfying the condition (3), ~~shortening of a distance from a shoulder of the objective lens on a body to the intermediate image position, and~~ miniaturization of the equipment can be achieved with a shortened distance from the objective lens shoulder on the main body to the intermediate image position.

By satisfying the condition (4), ~~correction of~~ spherical aberration ~~generated by shortening a focal length,~~ and chromatic aberration on the axis, which are caused by a shortened focal length, can be ~~carried out well~~ well compensated for.

[0022]

By constituting ~~that an~~ the image forming lens ~~consists of~~ with two lens groups, ~~correction of aberration in a first group~~ compensation for aberrations can be carried out by ~~a~~ the rear group so that ~~the aberration~~ aberrations produced in the front group ~~may be~~ is offset, and a ~~suitable~~ laser ~~beam~~ microscope ~~is obtained by~~ that is suitable for observation ~~at a state~~ in vivo is achieved.

By satisfying the conditions (5) and (6), ~~correction of astigmatic~~ astigmatism, coma aberration and magnification chromatic aberration can be ~~carried out~~ well compensated for.

[0023]

In a constitution comprising a first multi-mode fiber which leads the excitation light from the laser light source section to the scanning means, and a second multi-mode fiber which leads fluorescence from the sample to the detection optical system, by satisfying the conditions (7) to (9), the ~~rate~~ proportion of the amount of detected fluorescence ~~detected~~ to the amount of excitation light from the light source becomes high, ~~and~~ so that brighter fluorescence ~~can be~~ is detected~~, and furthermore~~. Furthermore, picture information ~~of a~~ on the thickness direction from the sample can be obtained covering a predetermined thickness. Accordingly, operation performance in observation in the state ~~that~~ where the sample is alive (in vivo) is improved.

[0024]

By arranging the optical transmission means between the pupil projecting lens and the detection optical system, a degree of freedom can be given to arrangement of the optical system ~~of~~ in the main body that is from the pupil projecting lens to the objective lens, and of

the detection optical system, and a miniaturized optical system of the main body suitable for observation in a state (in vivo) of the sample can be constituted.

Further, if the optical transmission means which leads the excitation light from the laser light source section to the scanning means~~,~~ and the optical transmission means which leads the fluorescence from the sample to the detection optical system are ~~arranged by~~ composed of separate optical fibers, respectively, the detection optical system can avoid an influence ~~of the self-generated~~ thereon by auto-fluorescence, which is generated when the excitation light enters ~~into~~ the optical fiber, and thus can detect the fluorescence generated by the sample ~~can be detected~~ with high ~~precision~~ accuracy.

~~BRIEF EXPLANATION OF DRAWINGS~~

BRIEF DESCRIPTION OF SEVERAL VIEWS OF THE DRAWING

[0025]

Figure 1 is ~~an outline block~~ schematic configuration diagram showing one conventional example of a laser scan type ~~co-focal point~~ confocal fluorescence microscope.

Figure 2 is ~~an outline block~~ a schematic configuration diagram of a first embodiment of a laser scan type fluorescence microscope according to the present invention.

Figure 3 is a principal-part diagram for ~~explaining a principal part~~ showing ~~an outline~~ a schematic configuration of the optical system arranged ~~at~~ in the main body ~~portion in~~ of the microscope of Fig. 2.

Figure 4 is a diagram showing ~~an optical arrangement,~~ a configuration in which a laser light source section and a detection optical system are added to the optical system ~~of a~~ in the main body ~~portion~~ of the microscope shown in Fig. 3.

Figure 5 is a diagram showing ~~an outline optical arrangement~~ a schematic configuration of an optical system in a second embodiment of the laser scan type fluorescence microscope according to the present invention.

Figure 6 is a diagram showing ~~an outline optical arrangement~~ a schematic configuration of an optical system in a third embodiment of the laser scan type fluorescence microscope according to the present invention.

Figure 7 is a diagram showing ~~an outline optical arrangement~~ a schematic configuration of an optical system in a fourth embodiment of the laser scan type fluorescence microscope according to the present invention.

Fig. 8 is a sectional ~~diagram~~ view taken along the optical axis for showing an optical arrangement ~~developed along an optical axis~~ of a pupil projection optical system and an

objective optical system ~~and a concerning the~~ of a first embodiment, used in the laser scan type fluorescence microscope of the present invention.

Fig.9 is a sectional ~~diagram~~ view taken along the optical axis for showing an optical arrangement ~~developed along an optical axis~~ of a pupil projection optical system and an ~~object~~ objective optical system of ~~the third~~ a second embodiment ~~according to~~, used in the laser scan type fluorescence microscope of the present invention.

Fig.10 is a sectional ~~diagram~~ view taken along the optical axis for showing an optical arrangement ~~developed along an optical axis~~ of ~~an~~ a pupil projection optical system and an ~~object~~ objective optical system of ~~the~~ a third embodiment ~~according to~~, used in the laser scan type fluorescence microscope of the present invention~~;~~.

~~BEST MODE FOR CARRYING OUT THE INVENTION~~
DETAILED DESCRIPTION OF THE INVENTION

[0026]

Figure 2 is ~~an outline block~~ a schematic configuration diagram of the first embodiment of ~~a~~ the laser scan type fluorescence microscope according to the present invention. Figure 3 is a principal-part diagram for ~~explaining a principal part~~ showing ~~an outline~~ a schematic configuration of an optical system arranged ~~at~~ in the main body ~~portion in~~ of the microscope of Fig. 2. Figure 4 is a diagram showing ~~an optical arrangement,~~ a configuration in which a laser light source section and a detection optical system are added to the optical system of ~~a~~ the main body ~~portion~~ of the microscope shown in Fig. 3.

The laser scanning fluorescence microscope of the first embodiment comprises, in a main body 11 of the microscope, a laser light source section 1, an exchangeable objective lens ~~unit~~ 2, a scanner section 3 as a scanning means, a lens unit 4 ~~equipped with~~ including a pupil projection lens 6 and an image forming lens ~~unit~~ 5, and a detection optical system 7 ~~in a main body 11 of a microscope~~.

It is desirable to use a semiconductor laser in ~~a~~ the laser light source section 1 since ~~a~~ the main body ~~portion~~ 11 of the microscope can be miniaturized by using it.

The objective lens ~~unit~~ 2 ~~is constituted by the objective lens optical system 8~~ forms, together with the image forming lens unit 5, an objective optical system 8. The objective ~~lens~~ optical system 8 has a function ~~which condenses~~ of condensing excitation light from the laser light source section 1 on a sample 10 on a stage 9. Moreover, the objective lens ~~unit~~ 2 is ~~constituted~~ configured so that ~~a~~ its back~~side~~ focal position ~~may become~~ is made conjugate ~~by~~

with a ~~neighborhood~~ position ~~of~~ near the scanner section 3 by the image forming lens ~~unit~~ 5 and a pupil projection lens 6. The image forming lens ~~unit~~ 5 has a function ~~which forms~~ of forming an intermediate image of the sample 10.

[0027]

The pupil projection lens 6 is arranged between the scanner section 3 and the objective ~~lens~~ optical system 8.

A detection optical system 7 has a barrier-filter 7a, a lens 7b, a ~~co-focal-point~~ confocal pinhole 7c, and a light receiving optical ~~sensors~~ sensor 7d, and it is ~~constituted~~ configured so that ~~the~~ fluorescence ~~which is emanated~~ emanating from the sample 10 and transmitted through the objective ~~lens~~ optical system 8 and the pupil projection lens 6 ~~may be~~ is detected by the light receiving optical sensor 7d.

The laser light source section 1 has a laser light source 1a and a collimating optical system~~, which consists of laser light source 1a, and Lenses~~ that is composed of lenses 1b and 1d and a pinhole 1c.

Between the scanner section 3 and the detection optical system 7, a dichroic mirror 16 is arranged for leading fluorescence from the sample 10 to the detection means 7, while leading excitation light from the light source section 1 to the sample ~~10 is arranged~~.

A laser drive section 14 ~~that drives emission of laser light from the laser light source 1a~~ is connected ~~to~~ with the laser light source section 1 for driving emission of laser light from the laser light source 1a.

A focusing mechanism ~~portion~~ section 12 for focusing the objective lens ~~unit~~ 2 is arranged on the main body ~~portion~~ 11 of the microscope.

In addition, the laser scan type fluorescence microscope has ~~a~~ an x-y-θ main ~~part~~ body moving mechanism 13 for ~~adjusting~~ performing positional adjustment of the main body 11 of the microscope, in two dimensions and with respect to an angle θ ~~of observation to a~~ at which the specimen is observed ~~and a position in directions of two dimensions with respect to the main body portion 11 of the microscope~~.

The laser scan type fluorescence microscope is connected with a processing control means 15, such as a personal computer and the like. The processing control means 15 is ~~constituted~~ constructed and arranged to carry out ~~a~~: wavelength control of laser light emanating from the laser light source ~~emanated by driving~~ as driven by the laser drive section 14~~, a~~; wavelength selection of ~~the~~ dichroic mirrors, ~~the~~ filters, etc.~~,~~; control of a wavelength-separation element~~,~~; drive control of the laser drive section 14~~,~~; analysis and display control of detection information received by the light receiving optical sensor 7d of the detection

optical system 7~~,~~; drive control of the scanner section 3~~,~~; drive control of ~~a~~ the focusing mechanism ~~portion~~ section 12~~, and~~; drive control of the x-y-θ main ~~part~~ body moving mechanism 13; and so on.

[0028]

In the laser scan type fluorescence microscope of the first embodiment, the excitation light ~~emanated~~ emanating from the laser light source 1a is condensed on the pinhole 1c by the lens 1b, and is converted into a beam of parallel ~~light~~ rays by the lens 1d. Then, it is led to the scanner section 3 ~~through~~ via the diachronic mirror 16, and ~~luminous flux~~ the beam of rays is shifted ~~to two directions of two dimensions~~ two-dimensionally in reference to the optical axis by each rotation of Galvano mirrors 3a and 3b of the scanner section 3 ~~to the optical axis, and by condensing~~, to be condensed at the intermediate image position as a primary image through the pupil projection lens 6~~, image forming of the primary image is carried out~~. The excitation light, which is condensed at the intermediate image position, is ~~irradiated by~~ incident on the sample 10 through the image forming lens ~~unit~~ 5 and the objective lens ~~unit~~ 2 in a shape like a minute spot. At this time, the excitation light ~~irradiated to the tenth~~, with which the surface of the sample ~~scanned~~ 10 is irradiated, is made to scan by the scanner section 3.

[0029]

The back~~side~~ focal position of the objective lens ~~unit~~ 2 is projected near the scanner section 3 by the image forming lens ~~unit~~ 5 and the pupil projection lens 8.

The fluorescence excited on the sample 10 by ~~irradiating~~ irradiation with the excitation light is led to the detection optical system 7 ~~through an~~ via the objective lens ~~unit~~ 2, the image forming lens ~~unit~~ 5, the pupil projection lens 6, the scanner section 3, and the dichroic mirror 16. Then, only the fluorescence transmitted through the ~~co-focal point~~ confocal pinhole 7c via the barrier filter 7a and the lens 7b is detected by the light receiving optical sensor 7d, such as a photo multiplier.

[0030]

Here, the laser scan type fluorescence microscope of the first embodiment is ~~constituted~~ configured to satisfy the following condition~~;~~:

$$0.15 \leq D/L \leq 0.5 \quad \ldots(1)$$

where D is a ~~co-focal length~~ parfocal distance of the objective lens ~~unit~~ 2, and L is a distance from the surface of the sample 10 to the ~~conjugate~~ position E ~~of~~ conjugate with the ~~backside~~ back focal position of the objective lens ~~unit~~ 2 ~~arranged~~ and located near the scanning means (scanner section 3) ~~from the tenth surface of the sample~~.

[0031]

When the condition (1) is satisfied as mentioned in the laser scan type fluorescence microscope of the first embodiment, it becomes possible to shorten a distance from the scanning means 3 to the sample 10, and miniaturization of the equipment can be achieved.

If it exceeds the ~~maximum value~~ upper limit of the condition (1), the focal lengths of the image forming lens 5 and the pupil projection lens 6 become short, and an interval between the pupil projection lens 6 and the scanning means 3 becomes short too much, and an interference occurs.

On the other hand, if it is less than the lower limit of the condition (1), the full length from the sample 10 to the scanning means 3 becomes long too much, and the miniaturization of the equipment becomes difficult.

[0032]

Figure 5 is a diagram showing ~~an outline optical arrangement~~ a schematic configuration of an optical system in ~~a~~ the second embodiment of the laser scan type fluorescence microscope according to the present invention. Here, the same ~~symbol is~~ symbols are used to show ~~a component~~ components having the same composition as in the first embodiment.

The laser scan type fluorescence microscope of the second embodiment is a modification of the first embodiment, where a dichroic mirror 20, a lens 19, an optical transmission means 18, a lens 17, and the dichroic mirror 16 are arranged between ~~the~~ a light source section ~~1~~ 1' and the scanning means 3.

~~An~~ The optical transmission means 18 ~~consists~~ is composed of an optical fiber, such as a single mode fiber or a multi-mode fiber. Since an end surface of the optical transmission means 18 is conjugate ~~to~~ with a specimen surface position and a core diameter of the end surface of the fiber serves as a ~~co-focal point~~ confocal pinhole, ~~the~~ a pinhole 1c of a ~~light source section~~ light source section 1' and ~~the~~ a pinhole 7c of a second detector 7' may be ~~arranged off~~ removed from an optical path~~,~~ or ~~the diameter~~ may ~~be big enough~~ have sufficiently large diameters in reference to ~~a~~ the diffraction diameter. When ~~an~~ the optical transmission means 18 is a multi-mode fiber, ~~a co-focal point effect becomes weaker since an a fiber~~ its large core diameter ~~becomes large~~ in reference to ~~a~~ the diffraction ~~core~~ diameter weakens the confocal effect, but it ~~is~~ makes it possible to pick up a fluorescence image brightly. Therefore, it is ~~good~~ preferred to choose a fiber according to ~~an~~ the purpose of observation ~~purpose~~. When performing ~~a~~ non-confocal, normal fluorescence observation ~~which is not a co-focal point by~~ using a multi-mode fiber for ~~an~~ the optical transmission

means 18~~,~~ it is ~~good constitute that~~ preferred to remove the pinhole 1c of the ~~light source section~~ light source section 1'~~,~~ and the pinhole 7c of the second detector 7' ~~are not arranged in~~ from the optical path.

[0033]

The dichroic mirror 20 is ~~constituted so that~~ configured to lead fluorescence from the sample 10 ~~may be led~~ to the second detection optical system 7', while leading the excitation light from the light source section 1' to the sample 10.

And ~~it is constituted~~ the configuration is made so that, via the optical transmission means 18, the fluorescence from the sample 10 ~~which transmitted~~ through the lens 17 is led to the second detection means ~~7~~ 7', while excitation light from the laser light source section ~~1~~ 1' is led to the scanning means 3 ~~through the optical transmission means 18~~.

In ~~an~~ the embodiment of Fig. 4, as for the ~~light source section~~ light source section 1', ~~two or more the~~ a plurality of sets each including a light source 1a to ~~the~~ a lens 1d are prepared, and ~~a dichroic mirror~~ dichroic mirrors 1e ~~is~~ are arranged accordingly.

[0034]

If an optical transmission means is arranged between the ~~laser light source section 1~~ laser light source section 1' and the scanning means 3 as shown in the laser scan type fluorescence microscope of the second embodiment, it becomes possible to give a degree of freedom to arrangement of ~~an~~ the optical system ~~of~~ in the main body ~~portion~~ of the equipment from the scanner section 3 to the objective lens ~~unit~~ 2 and the laser light source section ~~1~~ 1'. For this reason, ~~an~~ the optical system ~~of a~~ in the main body ~~portion~~ of ~~a~~ the microscope can be miniaturized in a suitable size for observation in a state of a sample being alive (in vivo).

In the second embodiment, if a near-infrared Femto-second pulsed laser is used for the laser light source 1', it is possible to make observation ~~becomes possible~~ using the microscope as a fluorescence microscope ~~of~~ with multiphoton excitation.

In this case, it is ~~good enough that~~ preferred to use the detector 7 ~~is used~~ as a detector ~~of the~~ for fluorescence ~~having~~ generated by multiphoton excitation, ~~and a~~ to appropriately select the spectrum characteristic of the dichroic mirrors 1e, 16, and 20 ~~are selected~~, and to make pinhole diameters of the pinholes 1c and 7c ~~are made~~ sufficiently larger ~~enough~~ than a diffraction ~~core,~~ diameter or ~~they are arranged off~~ remove the pinholes from the optical path.

[0035]

Fig. 6 is a diagram showing an outline composition of the optical system in the third embodiment of the laser scan type fluorescence microscope concerning the present invention.

Here, the same ~~symbol is~~ symbols are used to show ~~a component~~ components having the same composition as in the second embodiment.

The laser scan type fluorescence microscope of the third embodiment is a modification of the second embodiment~~. A~~, where a lens 22, the optical transmission means 21 which ~~consists~~ is composed of an optical fiber~~,~~ such as a single mode fiber or a multi-mode fiber, and a lens 23 are arranged between ~~a~~ the dichroic mirror 16 and ~~a~~ the detection optical system 7'. And ~~it is constituted~~ the configuration is made so that, while the excitation light from ~~light-source-section~~ a light source section 1" is led to the sample 10 via the optical transmission means 18, the fluorescence from the sample 10 ~~may be~~ is led to the second detection optical system 7' via the optical transmission means 21. As ~~shown~~ in the second embodiment, since end surfaces of optical fibers of the optical transmission means 18 and 21 are conjugate ~~to~~ with a specimen surface position and~~,~~ core diameters of the end surfaces of the fibers serve as ~~a co-focal point pinhole~~ confocal pinholes, ~~the~~ a pinhole 1c of ~~a light-source-section~~ the light source section 1" and the pinhole 7c of ~~a~~ the detector 7' may be ~~arranged off~~ removed from the optical path~~,~~ or ~~the diameter~~ may ~~be big enough~~ have sufficiently large diameters in reference to the diffraction diameter. When the optical transmission means 18 and 21 are multi-mode fibers, ~~a co-focal point effect becomes weaker since a fiber~~ their large core ~~diameter becomes large~~ diameters in reference to ~~a~~ the diffraction ~~core~~ diameter weaken the confocal effect, but ~~it is~~ they make it possible to pick up a fluorescence image brightly. Therefore, it is ~~good~~ preferred to choose a fiber according to ~~an~~ the purpose of observation ~~purpose~~. When carrying out ~~a~~ non-confocal, normal fluorescence observation ~~which is not a co-focal point, by~~ using ~~a~~ multi-mode ~~fiber~~ fibers for the optical transmission means 18 and 21, it is ~~good to constitute that~~ preferred to remove the pinhole 1c of the ~~light-source-section~~ light source section 1"~~,~~ and the pinhole 7c of the detector 7' ~~are not arranged in~~ from the optical path.

It is desirable to constitute such way as mentioned above, because ~~an~~ the optical system ~~of~~ in the main ~~part~~ body of ~~a~~ the microscope equipment can be miniaturized much more.

~~As shown in the laser scan type fluorescence microscope of the third embodiment, if~~ If an optical fiber constituting the optical transmission means 18, which leads the excitation light from ~~light-source-section~~ the light source section 1" to ~~a~~ the scanner section 3, and an optical fiber constituting the optical transmission means 21, which leads the fluorescence from ~~a~~ the sample 10 through the pupil projection lens 6 to the second detection optical system 7', are separately arranged, respectively, the second detection optical system 7' can

avoid an influence ~~of the self-generated~~ thereon by auto-fluorescence, which is generated when the excitation light enters ~~into~~ the optical fiber 18, and thus can detect the fluorescence generated by the sample 10 ~~can be detected~~ with high ~~precision~~ accuracy.

In the laser scan type fluorescence microscope of the third embodiment, when the optical transmission means 18 and 21 are ~~constituted by a~~ composed of multi-mode ~~fiber~~ fibers, it is desirable to satisfy the following conditions (7) to (9).

$$2 \leq \Phi em/\Phi ex \leq 12 \quad \ldots(7)$$

$$0.61 \times (\lambda ex/NAex) < \Phi ex \quad \ldots(8)$$

$$0.61 \times (\lambda em/NAem) < \Phi em \quad \ldots(9)$$

where Φex is a core diameter of the multi-mode fiber 18, Φem is a core diameter of the multi-mode fiber 21, NAex is ~~an~~ a numerical aperture ~~size of incidence~~ where light ~~to~~ is incident on the multi-mode fiber 18 ~~by~~ via the lens 19, λex is an excitation wavelength, NAem is ~~an~~ a numerical aperture ~~size of incidence~~ where light ~~to~~ is incident on the multi-mode fiber 21 ~~by~~ via the lens 22, and λem is a fluorescence wavelength.

[0037]

If the conditions (7) to (9) are satisfied, the ~~rate~~ proportion of the amount of detected fluorescence ~~detected~~ to the amount of excitation light from the light source becomes high, ~~and possible to pick up~~ so that a bright fluorescence image ~~brightly, and to obtain~~ is obtained. In addition, since a predetermined amount of picture information from the sample with respect to thickness direction~~. Therefore~~ can be obtained, operation performance is improved in observation in the state ~~that~~ where the sample is alive (in vivo).

If it is less than the lower limit of the condition (7), since the ~~rate~~ proportion of the detected fluorescence ~~detected~~ to the excitation light from the light source becomes low, ~~and~~ the acquired fluorescence image ~~obtained becomes dark,~~ is darkened and the picture information ~~of~~ on the thickness direction of the sample extremely decreases ~~very much~~. Therefore, the operation performance ~~at the time of~~ in observation worsens.

On the other hand, if it exceeds the ~~maximum value~~ upper limit of the condition (7), ~~the~~ too much picture information covering the thickness direction of the sample ~~enters too much~~ is obtained, and a picture other than the fluorescence to be observed ~~can be seen too much~~ becomes too conspicuous. Therefore, it becomes difficult to carry out a fluorescence observation.

If conditions (8) and (9) are not satisfied, the excitation light to the sample becomes weak~~,~~ or the ~~fluoreseence~~ intensity of detected fluorescence ~~is dark and~~ becomes low, to extremely decreases the picture information ~~of~~ on the thickness direction of the sample

~~decreases very much~~. Therefore the operation performance ~~at the time of~~ in observation worsens.

It is more desirable if the following condition (7-1) is satisfied.

$$4 \leq \Phi em/\Phi ex \leq 10 \quad \ldots(7\text{-}1)$$

~~the~~ The composition of the laser scan type fluorescence microscope which satisfies the conditions (7) to (9) mentioned above of the third embodiment is applicable also to a general laser scan type fluorescence microscope which does not satisfy the condition (1).

[0038]

Fig. 7 is a diagram showing an outline composition of the optical system in the fourth embodiment of the laser scan type fluorescence microscope according to the present invention. Here, the same symbol is used to show a component having the same composition in the first embodiment.

The laser scan type fluorescence microscope of the fourth embodiment is a modification of the first embodiment, where a ~~light-source-section~~ light source section 1''' ~~consists~~ is composed of a laser light source 1a and a lens 1d. The lens 22, the optical transmission means 21, the lens 23, and the detection optical system 7" are arranged at an opposite side of a Galvano mirror 3b which is separated from the dichroic mirror 16.

The optical transmission means 21 ~~consists~~ is composed of an optical fiber, such as a single mode fiber or a multi-mode fiber. An optical fiber end surface of the optical transmission means 21~~,~~is conjugate ~~to~~ with a specimen surface position, and since a core diameter of ~~a~~ the fiber end surface serves as a ~~co-focal-point~~ confocal pinhole, the pinhole 1c''' of the light source section 1 and the pinhole 7c of the detector 7' may be ~~arranged off~~ removed from the optical path~~,~~ or may have a larger diameter ~~to a~~ in reference to the diffraction diameter. If the optical transmission means 21 is a multi-mode fiber, it is possible to pick up fluorescence image brightly, although ~~a co-focal-point~~ the confocal effect becomes weaker as a fiber core diameter becomes large in reference to ~~a~~ the diffraction diameter~~,~~. Therefore, it is ~~good~~ preferred to choose a fiber according to ~~an~~ the purpose of observation ~~purpose~~.

[0039]

In the laser scan type fluorescence microscope according to the present invention shown by these embodiments, parallel luminous flux deflected by the scanning means (scanner section 3) is relayed to ~~an~~ the intermediate image position through the pupil projection lens 6.

Here, if a the pupil projection lens 6 is ~~constituted by~~ composed of two or more lenses, wherein a concave surface of the lens arranged nearest to the scanning-means side is directed to the scanning-means side, and a concave surface of the lens nearest to the middle-image side is directed to the middle-image side, correction of an optical performance in the intermediate image can be carried out well.

[0040]

In the laser scan type fluorescence microscope of the present invention shown by each of embodiments mentioned above, it is desirable to satisfy the following condition (2)~~:~~:

$$0.2 \leq Fe/D3 \leq 0.5 \quad \ldots(2)$$

where D3 is a distance from the ~~conjugate~~ position ~~of~~ conjugate with the pupil of the objective lens ~~unit~~ 2 and arranged near the scanning means to the intermediate image position of the image forming lens ~~unit~~ 5, and Fe is a focal length of the pupil projection lens 6.

[0041]

If condition (2) is satisfied, it is possible to shorten a distance from the scanning means to the intermediate image position, and to miniaturize the equipment, while keeping the optical performance of the pupil projection lens good.

If it exceeds the ~~maximum value~~ upper limit of the condition (2), the distance ~~of~~ from the scanning means ~~and~~ to the pupil projection lens becomes short, and ~~an interval of the pupil projection lens and the scanning means~~ accordingly a space between them becomes so short ~~too much, and it generates~~ to cause interference.

On the other hand, if less than the lower limit of the condition (2), it is difficult to miniaturize the equipment since the full length from the scanning means to the intermediate image position becomes too long ~~too much~~.

[0042]

In the laser scan type fluorescence microscope shown by each of the embodiments mentioned above, which comprises two or more lens groups, having at least one cemented lens of a positive lens and a negative lens, it is desirable to satisfy the following conditions (3) and (4)~~:~~:

$$0.4 \leq FTL/D1 \leq 1 \quad \ldots(3)$$

$$80 \leq \upsilon p \quad \ldots(4)$$

where υp is Abbe's Number of the positive lens in this cemented lens, FTL is a focal length of the image forming lens ~~unit~~ 5, and D1 is a distance from the position holding the objective lens unit 2 to the intermediate image position .

[0043]

If the condition (3) is satisfied, it becomes possible to shorten a distance from the ~~position holding~~ the objective lens shoulder to the intermediate image position, and to miniaturize the equipment. If the condition (4) is satisfied, ~~correction of~~ spherical aberration and axial chromatic aberration ~~at an axis~~ generated by ~~shortening~~ a shortened focal length are ~~carried out good~~ well compensated for, and ~~a co-focal point~~ confocal fluorescence observation from visible region to near-infrared region can be carried out.

When a ~~co-focal point~~ confocal fluorescence microscope is constituted in the present invention here, ~~none of~~ the spherical aberration and the axial chromatic aberration ~~at an axis is obtained~~ appear not as a picture ~~by~~ in the actually obtained picture ~~obtained in fact, and accordingly~~, but affects wave front aberration in each ~~of~~ wavelength and an observation domain ~~is affected~~. If the condition of wave front aberration of the optical system of the present invention is good, the picture obtained from the detector ~~becomes of~~ has high resolution and high S/N. Therefore, by correcting spherical aberration and off-axial aberration ~~off axis~~ in good condition, the wavefront aberration can be corrected good as a result. The same may be applied to each of aberrations described by the following-conditions.

If it exceeds the ~~maximum value~~ upper limit of the condition (3), an interval between the objective lens and the image forming lens becomes too short ~~too much, and it becomes difficult~~ to arrange a focusing mechanism ~~portion~~ by which the ~~free~~ working distance of the objective lens is changed.

On the other hand, if less than the lower limit of the condition (3), it becomes difficult for the full length from the sample to the scanning means to become long too much, and miniaturization of the equipment is difficult.

It is not desirable that it is less than the lower limit of the condition (4), If so, correction of the chromatic aberration at an axis and spherical aberration generated in the objective ~~lens~~ optical system becomes difficult, and accordingly the ~~co-focal point~~ confocal fluorescence observation from visible region to near-infrared light of the wavelength of the excitation light, becomes difficult.

[0044]

In the laser scan type microscope of each of embodiments mentioned above, if the image forming lens unit 5 ~~consists of~~ includes at least two lens groups~~, having at least~~ that are a front group at an intermediate image side~~,~~ and a rear group at an objective lens side, where ~~a lens group of~~ the front group ~~of an image forming lens unit 5~~ has at least one negative lens, it is desirable to satisfy the following conditions (5) and (6)~~:~~:

$$0.4 \leq D2/FTL \leq 1 \qquad \ldots(5)$$

$0.7 \leq FTL1/FTL \leq 1.5$ ...(6)

where FTL1 is a focal length of the rear group of the image forming lens ~~unit~~ 5, and D2 is an interval ~~of~~ between the front group of the image forming lens unit 5 and the rear group of the image forming lens unit 5.

[0045]

Thus, if the image forming lens unit 5 is ~~constituted by~~ composed of two lens groups, correction of the aberration can be carried out by the rear group so that the aberration in the front group may be negated, and a suitable laser scan type fluorescence microscope can be obtained by observation in vivo.

If conditions (5) and (6) are satisfied, it becomes possible to correct astigmatism, coma aberration and magnification chromatic aberration better.

It is not desirable if it exceeds the ~~maximum value~~ upper limit of the condition (5), since the ~~lens group of~~ lenses in the front group ~~becomes~~ are located so close to the intermediate image position ~~too much and this degrades~~ that the image quality ~~of the picture image by influence of blemish and garbage on~~ is degraded by flaws or dust on a lens surface~~,~~.

On the other hand, if less than the lower limit of the condition (5), chromatic aberration on the axis and coma aberration off the axis gets worse. Therefore, it is not desirable.

If it exceeds the ~~maximum value~~ upper limit of the condition (6), the power of the rear group becomes too weak ~~too much, and it becomes difficult~~ to carry out correction of color spherical aberration and coma aberration.

On the other hand, if it ~~exceeds~~ falls below the lower limit of the condition (6), the power of the rear group becomes too strong ~~too much, and~~ to achieve correction of chromatic aberration on the axis and coma aberration ~~becomes difficult~~.

On page 27, between lines 14 and 15, please insert the following new text:

$D = d_{24} + d_{25} + \ldots + d_{44}$ (r_{24} to r_{44})

$D1 = d_{14} + d_{15} + \ldots + d_{23}$ (r_{14} to r_{24})

$D2 = d_{18}$ (r_{18} to r_{19})

$D3 = d_1 + d_2 + \ldots + d_{13}$ (r_1 to r_{14})

On page 30, between lines 16 and 17, please insert the following new text:

$D = d_{24} + d_{25} + ... + d_{44}$ (r_{24} to r_{44})

$D1 = d_{14} + d_{15} + ... + d_{23}$ (r_{14} to r_{24})

$D2 = d_{18}$ (r_{18} to r_{19})

$D3 = d_1 + d_2 + ... + d_{13}$ (r_1 to r_{14})

On page 33, between lines 19 and 20, please insert the following new text:

$D = d_{25} + d_{26} + ... + d_{45}$ (r_{25} to r_{45})

$D1 = d_{15} + d_{16} + ... + d_{24}$ (r_{15} to r_{25})

$D2 = d_{19}$ (r_{19} to r_{20})

$D3 = d_1 + d_2 + ... + d_{14}$ (r_1 to r_{15})